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14. ABSTRACT
We hypothesize that BRCA2 facilitates the formation and/or the function of the ternary ISGF3 complex and thus, functional BRCA2 protein is essential for the antiproliferative effects of type I interferons against human breast tumor cells. S**pecific aims: (A)** To evaluate further the structural and functional interactions of BRCA2 with the members of the ISGF3 complex (STAT1, STAT2 and IRF9) in the human breast cells. **(B)** To evaluate the antiproliferative effects of BRCA2 over expression in the human breast cells with or without knock down of the IRF9 protein by RNA interference. **(C) To e**valuate the antiproliferative effects of type I interferons against tumors developed by BRCA2 positive and BRCA2 negative human breast tumor cells in the nude mice xenograft model.

15. SUBJECT TERMS
Breast cancer, BRCA2, Interferon alpha, Interferon beta, STAT1, STAT2, IRF9, ISGF3

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INTRODUCTION

BRCA2, a tumor suppressor whose inactivation is associated with hereditary breast and ovarian cancer predisposition, is essential for DNA repair in mammalian cells [1-5]. BRCA2-deficient cells are defective in the repair of DNA double-strand breaks by error-free homologous recombination [5, 6], allowing error-prone repair processes to create gross chromosomal re-arrangements that may promote carcinogenesis [6]. The role of BRCA2 in homologous recombination has been linked to its functions in the regulation of RAD51, a RecA-related recombinase that forms the nucleoprotein filaments on damaged DNA that are crucial to recombinational repair [7, 8]. BRCA2 binds directly to RAD51 through 6 of the 8 BRC repeats, ~30 amino acid motifs encoded within the central exon 11 region of all known mammalian BRCA2 genes [6, 9-12]. In addition to its role in DNA double-strand break repair, BRCA2 also plays a role in stabilization of stalled DNA replication forks, cytokinesis, transcription regulation, mammalian gametogenesis, centrosome duplication, and suppression of cell proliferation [6]. However, how BRCA2 mutations predispose women specifically to breast and ovarian cancer remains undefined. One of the possible pathways for the antiproliferative effect of BRCA2 is mediated through the MAGE-D1 protein [13]. BRCA2 binds and stabilizes MAGE-D1, a member of the MAGE gene family of proteins. Expression of BRCA2 and MAGE-D1 synergistically suppresses cell proliferation independently of the p53 pathway. MAGE-D1 is a downstream target of BRCA2 and that BRCA2 suppresses cell proliferation via stabilizing MAGE-D1 [13].

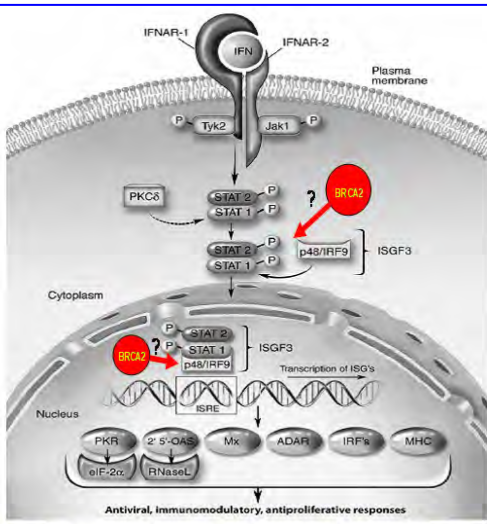


Fig. 1. Type I interferon (IFN) pathway (1-6). The postulated roles of BRCA2 (in red) are indicated.

We found that transient ablation of the breast cancer susceptibility gene BRCA2 in the human breast cells (Fig. 1) impairs the expressions of many type I interferon regulated genes (Table 1). Thus, it appears that type I interferons need functional BRCA2 for their actions [14]. Thus, negative regulation of IFN-induced genes in BRCA2-ablated breast cells may reflect another growth regulatory role of BRCA2.

The interferons (IFNs), in addition to their well-known antiviral activities, have important roles in the control of cell proliferation and are effective agents for the treatment of malignant diseases. IFNs not only regulate cell growth and division but also influence cell survival through their effects on apoptosis.   and   interferons are type I IFN proteins with antitumor activity [15, 16]. They down regulate oncogene expression and induce tumor suppressor genes, which result in antiproliferative activity. The classic pathway induced by type I IFNs involves the interaction of the IFN with two-receptor subunits, IFNAR-1 and -2, which are associated with TYK-2 and JAK-1, respectively [17-19]. TYK-2 and JAK-1 phosphorylate tyrosine residues on the receptor that provide docking sites for the src-homology-2 (SH2) domains of STATs in a cell type specific manner [15, 16]. Once phosphorylated, STATs are released from the receptor and form heterodimers. In response to Type I IFNs, STAT2 is recruited to the IFNAR1 chain, where it is phosphorylated by TYK-2 and serves as a lure for STAT1 [15, 16]. Once released from the receptor, the resulting STAT1:STAT2 heterodimer associates with IRF9, a DNA binding protein (also called p48), forming a complex named IFN-stimulated gene factor-3 (ISGF3). After formation, ISGF3 translocates to the nucleus where it binds to the IFN-stimulated response elements (ISRE) upstream of IFN response genes and initiates transcription (Fig. 1).

We hypothesize that BRCA2 facilitates the formation and/or the function of the ternary ISGF3 complex and thus, functional BRCA2 protein is essential for the antiproliferative effects of type I interferons against human breast tumor cells.

Specific aims to verify the hypothesis are: (A) To evaluate further the structural and functional interactions of BRCA2 with the members of the ISGF3 complex (STAT1, STAT2 and IRF9) in the human breast cells. **(B)** To evaluate the antiproliferative effects of BRCA2 over expression in the human breast cells with or without knock down of the IRF9 protein by RNA interference. **(C)** To evaluate the antiproliferative effects of type I interferons against tumors developed by BRCA2 positive and BRCA2 negative human breast tumor cells in the nude mice xenograft model.

BODY

Task outlined in the approved Statement of Work for this period of the project

Task#1

To evaluate further the structural and functional interactions of BRCA2 with the members of the ISGF3 complex (STAT1, STAT2 and IRF9) in the human breast cells. (Months 1-18)

(a) Evaluation of requirements of the BRCA2 domains in the BRCA2/ISGF3 binding by pull down assays using three recombinant fragments (N-terminal, middle, and C-terminal of approximately equal lengths) of human BRCA2 protein liked to GST-tag. (Months 11-15).

(b) Further evaluation of direct binding between BRCA2 and ISGF3 components by yeast 2-hybrid analysis. (Months 6-18)

Task#2

To evaluate the antiproliferative effects of BRCA2 over expression in the human breast cells with or without knock down of the IRF9 protein by RNA interference. (Months 16-30)

(a) Generation of MDA-MB-231 and BT-549 cell derivatives inducibly over expressing BRCA2 and their characterization. (Months 16-19).

(b) Knock down of IRF9 gene expression in the control and the BRCA2-over expressing cells. (Months 19-22).
(c) Evaluation of the levels of BRCA2 and IRF9 mRNAs and proteins in the cells by RT-PCR and Western blotting, respectively. (Months 22-23)

1. We evaluated the ability of different domains of human BRCA2 protein in pulling down the components of the BRCA2/ISGF3 complex. Human BRCA2 is a large protein (3418 amino acids). We have amplified 5 consecutive fragments from the human BRCA2 ORF (in plasmid pCINBRCA2WT). There are five peptide fragments: the first 4 fragments are from the N-terminal end and have 683 amino acids (ORF ~2055 bp with start and stop codons) and the 5th C-terminal fragment has 686 amino acids. We have cloned these PCR amplified products into N-terminal GST-tagged protein expression vector [pFN2K (GST) Flexi® Vector]. We expressed these N-terminal GST-tagged BRCA2 peptide fragments in human breast cells (Fig. 2). By GST-pull down assays we found that only the N-terminal 683 amino acid fragment of BRCA2 was able to pull down all three components of the ISGF3 complex (Fig. 2). We conclude that BRCA2 interacts with the ISGF3 complex perhaps through the N-terminal domains. As it does not interact with its BRC repeats with ISGF3, ISGF3 will not compete with RAD51.

BRCA2-F1
MPIGSKERPTFFEIFKTRCNKADLGPISLNWFEELSSEAPPYNSEPAEESEHKNNNYEPNLFKTPQRKPSYNQLASTPI
IFKEQGLTLPLYQSPVKELDKFKLDLGRNVPNSRHKSLRTVKTKMDQADDVSCPLLNSCLSESPVVLQCTHVTPQRDK
SVVCGSLFHTPKFVKGRQTPKHISESLGAEVDPDMSWSSSLATPPTLSSTVLIVRNEEASETVFPHDTTANVKSYFSN
HDESLKKNDRFIASVTDSENTNQREAASHGFGKTSGNSFKVNSCKDHIGKSMPNVLEDEVYETVVDTSEEDSFSLCF
SKCRTKNLQKVRTSKTRKKIFHEANADECEKSKNQVKEKYSFVSEVEPNDTDPLDSNVANQKPFESGSDKISKEVVP
SLACEWSQLTLSGLNGAQMEKIPLLHISSCDQNISEKDLLDTENKRKKDFLTSENSLPRISSLPKSEKPLNEETVVNKR
DEEQHLESHTDCILAVKQAISGTSPVASSFQGIKKSIFRIRESPKETFNASFSGHMTDPNFKKETEASESGLEIHTVCSQ
KEDSLCPNLIDNGSWPATTTQNSVALKNAGLISTLKKKTNKFIYAIHDETSYKGKKIPKDQKSELINCSAQFEANAFEAP
LTFANADSGLLHSSVKRSCSQNDSEEPTLSLTSSFGTILRKCSRNETCSNNTVIS

BRCA2-F2
QDLDYKEAKCNKEKLQLFITPEADSLSCLQEGQCENDPKSKKVSDIKEEVLAAACHPVQHSKVEYSDTDFQSQKSLLY
DHENASTLILTPTSKDVLSNLVMISRGKESYKMSDKLKGNNYESDVELTKNIPMEKNQDVCALNENYKNVELLPPEKY
MRVASPSRKVQFNQNTNLRVIQKNQEETTSISKITVNPDSEELFSDNENNFVFQVANERNNLALGNTKELHETDLTCV
NEPIFKNSTMVLYGDTGDKQATQVSIKKDLVYVLAEENKNSVKQHIKMTLGQDLKSDISLNIDKIPEKNNDYMNKWAGL
LGPISNHSFGGSFRTASNKEIKLSEHNIKKSKMFFKDIEEQYPTSLACVEIVNTLALDNQKKLSKPQSINTVSAHLQSSV
VVSDCKNSHITPQMLFSKQDFNSNHNLTPSQKAEITELSTILEESGSQFEFTQFRKPSYILQKSTFEVPENQMTILKTTS
EECRDADLHVIMNAPSIGQVDSSKQFEGTVEIKRKFAGLLKNDCNKSASGYLTDENEVGFRGFYSAHGTKLNVSTEAL
QKAVKLFSDIENISEETSAEVHPISLSSSKCHDSVVSMFKIENHNDKTVSEKNNKCQLILQNNIEMTTGTFVEEITENYK
RNTENEDNKYTAASRNSHNLEFDGSDSSKNDTVCIHKDETDLLFTDQHNICL

BRCA2-F3
KLSGQFMKEGNTQIKEDLSDLTFLEVAKAQEACHGNTSNKEQLTATKTEQNIKDFETSDTFFQTASGKNISVAKESFN
KIVNFFDQKPEELHNFSLNSELHSDIRKNKMDILSYEETDIVKHKILKESVPVGTGNQLVTFQGQPERDEKIKEPTLLGF
HTASGKKVKIAKESLDKVKNLFDEKEQGTSEITSFSHQWAKTLKYREACKDLELACETIEITAAPKCKEMQNSLNNDKN
LVSIETVVPPKLLSDNLCRQTENLKTSKSIFLKVKVHENVEKETAKSPATCYTNQSPYSVIENSALAFYTSCSRKTSVSQ
TSLLEAKKWLREGIFDGQPERINTADYVGNYLYENNSNSTIAENDKNHLSEKQDTYLSNSSMSNSYSYHSDEVYNDS
GYLSKNKLDSGIEPVLKNVEDQKNTSFSKVISNVKDANAYPQTVNEDICVEELVTSSSPCKNKNAAIKLSISNSNNFEV
GPPAFRIASGKIVCVSHETIKKVKDIFTDSFSKVIKENNENKSKICQTKIMAGCYEALDDSEDILHNSLDNDECSTHSHKV
FADIQSEEILQHNQNMSGLEKVSKISPCDVSLETSDICKCSIGKLHKSVSSANTCGIFSTASGKSVQVSDASLQNARQV
FSEIEDSTKQVFSKVLFKSNEHSDQLTREENTAIRTPEHLISQKGFSYNV

BRCA2-F4
VNSSAFSGFSTASGKQVSILESSLHKVKGVLEEFDLIRTEHSLHYSPTSRQNVSKILPRVDKRNPEHCVNSEMEKTCS
KEFKLSNNLNVEGGSSENNHSIKVSPYLSQFQQDKQQLVLGTKVSLVENIHVLGKEQASPKNVKMEIGKTETFSDVPV
KTNIEVCSTYSKDSENYFETEAVEIAKAFMEDDELTDSKLPSHATHSLFTCPENEEMVLSNSRIGKRRGEPLILVGEPSI

KRNLLNEFDRIIENQEKSLKASKSTPDGTIKDRRLFMHHVSLEPITCVPFRTTKERQEIQNPNFTAPGQEFLSKSHLYEH
LTLEKSSSNLAVSGHPFYQVSATRNEKMRHLITTGRPTKVFVPPFKTKSHFHRVEQCVRNINLEENRQKQNIDGHGSD
DSKNKINDNEIHQFNKNNSNQAAAVTFTKCEEEPLDLITSLQNARDIQDMRIKKKQRQRVFPQPGSLYLAKTSTLPRISL
KAAVGGQVPSACSHKQLYTYGVSKHCIKINSKNAESFQFHTEDYFGKESLWTGKGIQLADGGWLIPSNDGKAGKEEF
YRALCDTPGVDPKLISRIWVYNHYRWIIWKLAAMECAFPKEFANRCLSPERVLLQLKYRYDTEIDRSRRSAIKKIMERD
DTAAKTLVLCVSDIISLSANISETSSNKTSSADTQKVAIIELTDGWYAVKAQL

BRCA2-F5

DPPLLAVLKNGRLTVGQKIILHGAELVGSPDACTPLEAPESLMLKISANSTRPARWYTKLGFFPDPRPFPLPLSSLFSD
GGNVGCVDVIIQRAYPIQWMEKTSSGLYIFRNEREEEKEAAKYVEAQQKRLEALFTKIQEEFEEHEENTTKPYLPSRAL
TRQQVRALQDGAELYEAVKNAADPAYLEGYFSEEQLRALNNHRQMLNDKKQAQIQLEIRKAMESAEQKEQGLSRDV
TTVWKLRIVSYSKKEKDSVILSIWRPSSDLYSLLTEGKRYRIYHLATSKSKSKSERANIQLAATKKTQYQQLPVSDEILF
QIYQPREPLHFSKFLDPDFQPSCSEVDLIGFVVSVVKKTGLAPFVYLSDECYNLLAIKFWIDLNEDIIKPHMLIAASNLQW
RPESKSGLLTLFAGDFSVFSASPKEGHFQETFNKMKNTVENIDILCNEAENKLMHILHANDPKWSTPTKDCTSGPYTA
QIIPGTGNKLLMSSPNCEIYYQSPLSLCMAKRKSVSTPVSAQMTSKSCKGEKEIDDQKNCKKRRALDFLSRLPLPPPV
SPICTFVSPAAQKAFQPPRSCGTKYETPIKKKELNSPQMTPFKKFNEISLLESNSIADEELALINTQALLSGSTGEKQFIS
VSESTRTAPTSSEDYLRLKRRCTTSLIKEQESSQASTEECEKNKQDTITTKKYI

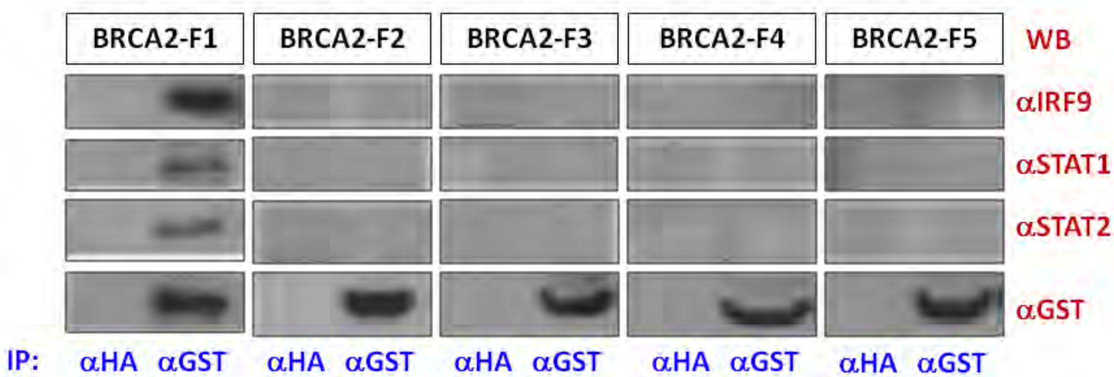
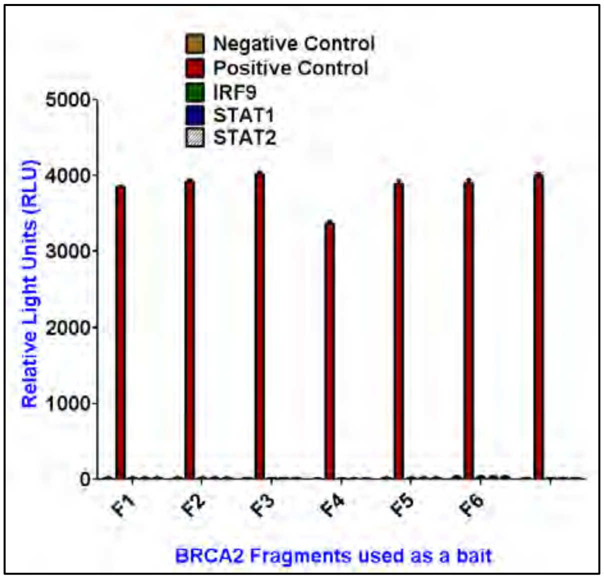


Fig. 2. Western blot analysis of the proteins immunopulled down with different fragments of human BRCA2 protein in MDA-MB-231 cells. HA antibody was used as a control in the pulled down experiment. Similar results were obtained with BT549 cells.

Fig. 1. Amino acid sequences of different fragments of human BRCA2 protein tested for their abilities to bind to the components of the ISGF3 complex.

2. Further evaluation of direct binding between BRCA2 and ISGF3 components by yeast 2-hybrid analysis. We employed BD Clontech Matchmaker Gold yeast two hybrid system for this purpose. We cloned the five BRCA2 ORF fragments described in the previous section into pGBKT7 DNA-BD cloning vector. Similarly we have cloned IRF9 and Stat-2 (wild type and Y690A mutant) ORFs into pGADT7 AD cloning vector. We are also cloned Stat-1 ORFs into pGADT7 vector. The yeast cells were co-transfected individually with pGADT7 fusion construct and pGBKT7 fusion construct. Yeast cells were plated onto synthetic dropout medium lacking leucine, tryptophan, and histidine in the presence of 5-bromo-4-chloro-3-indolyl-ft-D-galactopyranoside (X-α-Gal; Clontech) to select for yeast containing weaker interacting proteins. Yeast cells were also plated onto synthetic dropout medium lacking leucine, tryptophan, histidine, and adenine in the presence of 5-bromo-4-chloro-3-indolyl-ft-D-galactopyranoside (X-α-Gal; Clontech) to select for yeast containing stronger interacting proteins. The positive control (supplied with the reagent kit) used was SV40 T-antigen and p53, known to interact very strongly. The negative controls used were cells co-transfected with empty pGADT7/pGBKT7 vector, untransformed AH109 cells and singly transformed yeast cells. β-Galactosidase assays were done using the Beta Glo Assay reagents and protocols (Promega) to detect β-Galactosidase activity in the cotransfected yeast cell extracts. Yeast cells as well as the Beta-Glo reagent were brought to room temperature.



Equal volumes of the reagent and the yeast cell culture were added together. The sample contents were mixed for 30 seconds. Samples were then incubated for 30 min at room temperature and the luciferase activity was measured using a luminometer. Both the cell growth data (not shown) as well as the beta-glo assay data (Fig. 3) suggest that none of the five BRCA2 fragments directly binds to the individual component proteins of the ISGF3 complex in the yeast cells. These data may suggest that BRCA2 perhaps interacts with the ISGF3 complex once it is formed as a trimer.

Fig. 3. Beta-glo assay showing no direct binding of any of the five BRCA2 protein fragments with IRF9, STAT1 or STAT2 individually inside the yeast cells.

3. Generation of MDA-MB-231 and BT-549 cell derivatives inducibly over expressing BRCA2 and their characterization. We have generated lentiviral constructs for full length BRCA2 with a C-terminal FLAG tag, as described before for SLUG [20, 21]. We transfected MDA-MB-231 and BT549 cells constitutively expressing tet-repressor protein with this construct, selected for stable transfectants and then evaluated the mRNA and protein levels of recombinant BRCA2 in the presence or absence of doxycycline. We evaluated recombinant BRCA2 mRNA levels with primers designed from BRCA2 ORF and the FLAG epitope. These primers did not amplify the native BRCA2 in the cells. We performed real-time RT-PCR analysis for this evaluation. Total RNA was isolated from the cultured cells using TRIzol reagent (Invitrogen). The cDNA was synthesized from 1 µg of total RNA using the iScript cDNA Synthesis kit (Bio-Rad). Real-time PCR quantification was performed following standard protocols using Syber green dye (BioRad). RT-PCR was performed in the iCycler (BioRad): 95 °C for 10 min, 40 cycles of 15 s at 95 °C, 30 s at 51 °C, 30 s at 72 °C followed by 1 min at 95 °C, 30 s at 55 °C and 30 s at 95 °C. The fold change over control samples was calculated using *CT*, Δ*CT*, and ΔΔ*CT* values [20, 21]. β-Actin RNA was used as an endogenous control. For the evaluation of BRCA2 protein levels in the breast cancer cell lines by Western blotting with FLAG antibody, protein bands were developed using IR Dye 800 conjugated secondary antibody (LI-COR Biosciences), and visualized using LI-COR's Odyssey Infrared Imaging System. Quantitation and analysis of bands were performed using Odyssey's software. β-actin was used as normalization control. Figs. 4A and 4B

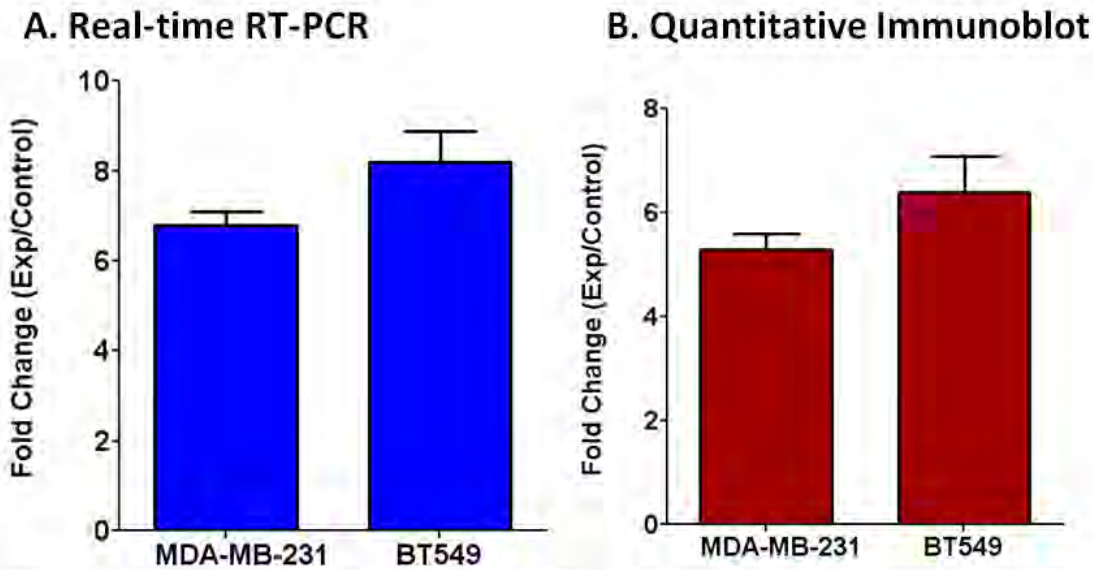


Fig. 4. Over expression of FLAG-tagged BRCA2 in MDA-MB-231 and BT549 cells. (A) Evaluation of recombinant BRCA2 mRNA levels by real-time RT-PCR. (B) Evaluation of recombinant BRCA2 protein by quantitative immunoblot analysis using FLAG antibody. Cells not treated with doxycycline were used as corresponding control. Results are mean $\pm$ SEM (n=6). The differences were statistically significant $p<0.001$.

show the over expression of recombinant BRCA2 mRNA and protein in these stably transfected cells.

4. Knockdown of IRF9 gene expression in the control and the BRCA2-over expressing cells. IRF9 siRNAs and corresponding control siRNAs were designed using the Block-IT RNAi designer software (Invitrogen) and purchased from Invitrogen. The nucleotide sequences of these siRNAs and respective control RNAs used in this study are as follows: Stealth961: 5´-GAGCUCUUCAGAACCGCCUACUUCU-3´/5´-AGAAGUAGGCGGUUCUGAAGAGCUC-3´; Control961: 5´-GAGUCCUGAAACCCGUCCAUUCUCU-3´/5´-AGAGAAUGGACGGGUUUCAGGACUC-3´; Stealth1025: 5´-CACCGAAGUUCCAGGUAACACUGAA-3´/5´-UUCAGUGUUACCUGGAACUUCGGUG-3´; Control1025: 5´-CACGAACUUGACAUGACAUCCGGAA-3´/5´-UUCCGGAUGUCAUGUCAAGUUCGUG-3´. Transfection of these siRNAs into the breast cells was done by lipofection using the Lipofectamine 2000 (Invitrogen) as per the manufacturer's instructions. Briefly, cells were transfected at ~50% confluence using 100 pmol siRNA in six well plates and whole-cell lysates were prepared 48 h after transfection. We isolated RNA from these cells using Trizol reagents (Invitrogen). Knockdown of the expressions of the target mRNAs by the experimental siRNA and the corresponding protein were verified by real-time RT-PCR and immunoblot analysis, respectively [20, 21] as described in the previous section. Our data (Fig. 5A and 5B) shows the successful knockdown of IRF9 mRNA (Fig. 5A) and protein (Fig. 5B) in the MDA-MB-231 and BT549 cells with the Stealth siRNAs used.

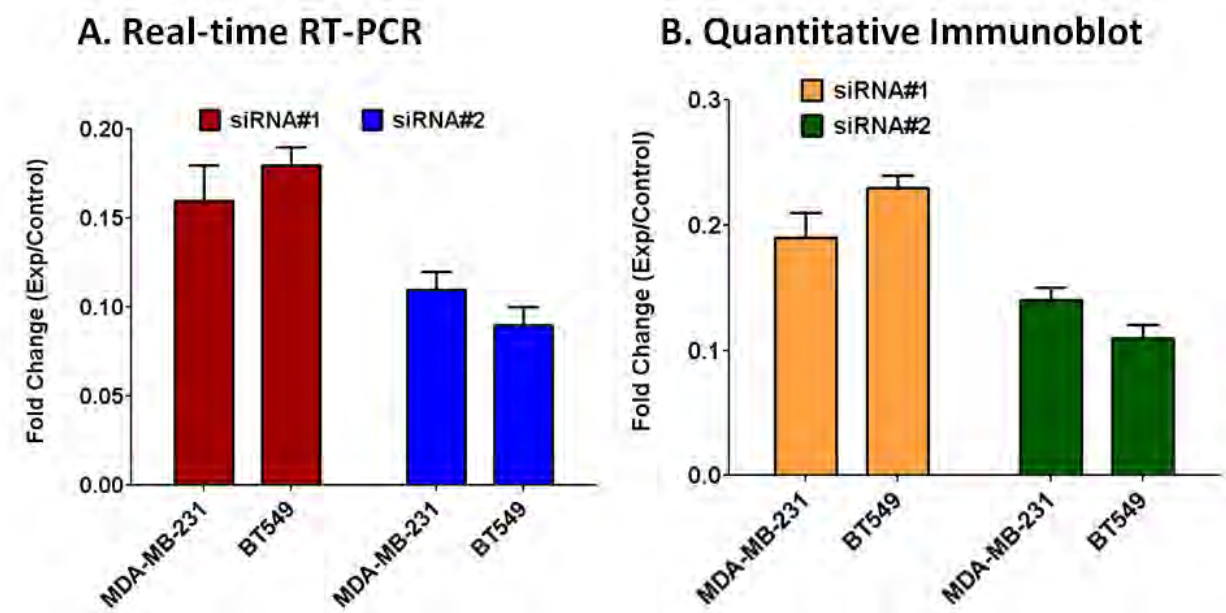


Fig. 5. Knockdown of IRF9 in MDA-MB-231 and BT549 cells using two different Stealth siRNAs. (A) Evaluation of IRF9 mRNA levels by real-time RT-PCR. (B) Evaluation of IRF9 protein by quantitative immunoblot analysis. Cells treated with control siRNA were used as corresponding control. Results are mean ± SEM (n=6). The differences were statistically significant $p<0.001$.

Fig. 6 shows the inhibition of the ISG15 gene promoter activity in the IRF9 knocked down cells. We evaluated ISG15 gene promoter activity in transiently transfected cells by dual luciferase assay [20, 21]. We PCR amplified human ISG15 gene promoter from total DNA isolated from MDA-MB-231 cells with specific primers. The amplified DNA was cloned into the pCR4.0/TOPO vector (Invitrogen) and subsequently subcloned into the Hind III/Pst I sites of pRL-Null vector (Promega). Cells were seeded on 24-well tissue culture plates in triplicate and allowed to grow overnight to reach 90% to 95% confluence. The following day cells were transfected with pGL3-Control and pRL-ISG15 promoter construct using Lipofectamine 2000 transfection reagent (Invitrogen). Forty-eight hours later, luciferase activities were measured using the Dual Luciferase Reporter Assay System (Promega) [20, 21]. *Renilla* Luciferase activity was normalized to firefly luciferase activity [20, 21].

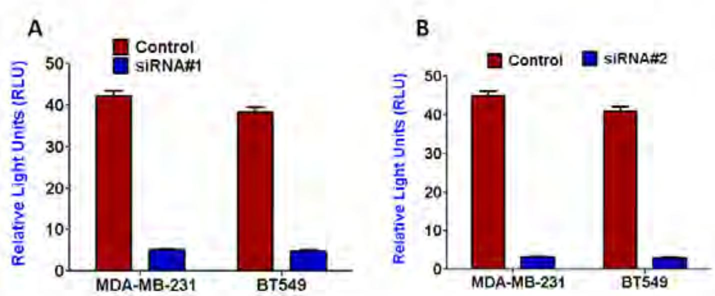


Fig. 6. Evaluation of the effect of IRF9 knockdown in two different human breast cancer cell lines on the ISG15 promoter activity. ISG15 gene expression is dependent upon the functional IRF9. Thus knockdown of IRF9 should decrease the activity of ISG15 gene promoter. (A) Data with Stealth RNA#1; (B) Data with Stealth RNA#2. Controls indicate corresponding control siRNAs. Results are mean ± SEM (n=6). The differences were statistically significant $p<0.001$.

Ongoing experiments:

(i) Determination of cell growth rate by thymidine incorporation assay, by colorimetric assay, clonogenic assay and flow cytometric analysis.

(ii) Evaluation of the tumorigenecity of the BRCA2 over expressing cells with or without knock down of IRF9.

Key Research Accomplishments

- N-terminal sequences of human BRCA2 protein appears to be involved in the direct binding of this protein with the ISGF3 protein complex as was revealed by pull down analysis.
- None of the fragments of BRCA2 protein could directly bind with individual components of the ISGF3 complex in the yeast 2-hybrid analysis, suggesting that BRCA2 perhaps binds with the ternary complex after it is formed.
- We were successful in over expressing FLAG-tagged BRCA2 protein in an inducible manner in MDA-MB-231 and BT549 cells.
- We successfully knocked down IRF9 in the breast cancer cells.
- Knock down of IRF9 negatively affected ISG15 gene promoter activity in the knocked down cells.

REPORTABLE OUTCOMES: We have not yet published or presented the research performed in this grant project. But the research performed in this project directly or indirectly contributed to the following publications and poster abstracts.

Publication:

1. Mittal, M. K., Myers, J. N., Bailey, C. K., Misra, S. and **Chaudhuri, G**. (2010) Mode of action of the retrogene product SNAI1P, a SNAIL homolog, in human breast cancer cells. ***Mol. Biol Report*** **37**, 1221-1227.

2. Misra, S. Sharma, S., Agarwal, A., Khedkar, S. V., Tripathi, M. K., Mittal M. K., and **Chaudhuri, G**. (2010) Cell cycle-dependent regulation of the bi-directional overlapping promoter of human BRCA2/ZAR2 genes in breast cancer cells. ***Molecular Cancer*** **9**, 50.

Meeting abstracts:

The current research on this project directly or indirectly affected the studies performed in the following poster presentations from our lab during 2009-2010:

1. Mittal, M. K., Singh, K. and **Chaudhuri, G.** (2009) Mechanisms of SLUG-induced drug resistance development in breast cancer cells. **Presented as poster at the 2009 San Antonio Breast Cancer Symposium (SABCS) held in Henry B. Gonzalez Convention Center, San Antonio, Texas, USA, December 9-13, 2009.**

2. Bailey, C. K., Mittal, Mukul, Misra, Smita and **Chaudhuri, G.** (2010) Reduction of the invasive phenotype of SNAI-over expressing human breast cancer cells by peptide aptamer-mediated inhibition of SNAI protein functions. **Presented at the AACR Annual meeting in Washington DC on April 17-21, 2010.**

3. Mittal, M. K., and **Chaudhuri, G.** (2010) Repression of alpha-, beta- and gamma-catenin gene expressions by SNAI2 in human breast cancer cells. **Presented at the AACR Annual meeting in Washington DC on April 17-21, 2010.**

4. Hall, Mack III, Misra, Smita, and **Chaudhuri, G.** (2010) Molecular analysis of the physical interactions of *Trypanosoma brucei* BRCA2 with different RAD51 isoforms. **Presented at the ASBMB Annual meeting in Anaheim, CA on April 24-28, 2010.**

5. Misra, Smita, and **Chaudhuri, G.** (2010) Regulation of BRCA2 gene expression through CpG methylation of its bi-directional promoter induced by endogenous siRNAs. **Presented at the ASBMB Annual meeting in Anaheim, CA on April 24-28, 2010.**

Conclusion:

BRCA2 interacts with the ISGF3 ternary complex through its N-terminal domain. We are currently evaluating the effect of IRF9 knockdown in breast cells on the tumor suppressive effects of BRCA2 protein.

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APPENDICES: None.